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Applicant: Pakola *et al.*

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Respectfully submitted,

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## 2. Patent application number

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0228409.9

06 DEC 2002

## 3. Full name, address and postcode of the or of each applicant (underline all surnames)

Thromb-X nv, Leopoldstraat 1 bus 21, 3000 Leuven

Patents ADP number (if you know it)

8311607002

If the applicant is a corporate body, give the country/state of its incorporation

Belgium

## 4. Title of the invention

Pharmacological vitreolysis

## 5. Name of your agent (if you have one)

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

Thromb-X nv

Care off:

Prof. Désiré José Collen

Collingham Garden 28

London SW5 0HN

Patents ADP number (if you know it)

8213118002

## 6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

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11.

Jean-Marie Stassen - Managing Director

I/We request the grant of a patent on the basis of this application.

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05/12/2002

12. Name and daytime telephone number of person to contact in the United Kingdom

Prof. Désiré José Collen

Tel. 020 72449405 fax 020 72449406

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**DUPLICATE****Pharmacological Vitreolysis**

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**Technical field**

This invention relates to a method of treating diseases of the eye or dysfunctions in the eye. It also involves enzyme preparations for therapeutic administration to the eyes of humans or other mammals. Specifically, this invention is directed to a method and composition which involves stabilised microplasmin (s-micro-Pm) and/or stabilised miniplasmin (s-micro-Pm) useful in the liquefaction of the vitreous, a normally clear jelly-like substance that fills the eye from the iris to the retina, and/or to disinsert the peripheral vitreous from the neurosensory retina (pharmacological vitrectomy). Vitrectomy involves the removal of the vitreous humor from the eye and possibly the replacement of the vitreous humor by a sterile isotonic solution. Vitrectomy may be necessary to remove the vitreous in order to reposition the retina and restore vision. Stabilised microplasmin or stabilised miniplasmin, delivered to the vitreous or to the vitreoretinal interface under stabilised form or after activation, can be used to induce posterior vitreous detachment and to assist mechanical vitrectomy or vitreoretinal surgery and to decrease viscosity of the vitreous.

**Background of the invention**

The vitreous is a clear, proteinaceous mass, which fills the posterior cavity of the eye between the lens and the retina. The vitreous is attached at its posterior face to the retina along the structure known as the internal limiting membrane. This site of attachment of the vitreous and the retina is termed the vitreoretinal junction and consists of a layer of basement membrane proximal to the retina and a layer of collagen fibrils proximal to the vitreous. It is well known that this vitreoretinal interface plays important roles in developing vitreoretinal pathologies that can lead to partial or complete blindness examples of which are retinal detachments, macular hole formation, macular traction syndromes including cystoid macular edema, and in retinopathy of prematurity.

~~Degenerative changes in the vitreous~~ are a precursor to posterior vitreous detachment.

Degeneration of the vitreous is part of the normal ageing process, but also may be induced by pathological conditions such as diabetes, Eales' disease and uveitis (Gloor, B. P., "The Vitreous", in Adler's Physiology of the Eye, C. V. Mosby, St. Louis, Mo., 1987). Because the vitreous is attached to the retina, the receding vitreous can precipitate a retinal tear, with subsequent detachment of the retina.

Certain pathological conditions of the eye are accompanied by the formation of new (abnormal) vessels on the surface of the retina--namely proliferative diseases. Posterior vitreous traction placed on new vessels causes rupture and bleeding. Proliferative retinal diseases thus are accompanied by both a high probability of retinal detachment as well as complications from bleeding resulting from the rupture of the newly formed blood vessels.

In the above mentioned disorders, vitrectomy, involving the removal of the vitreous humor from the eye and possibly the replacement of the vitreous humor by a sterile isotonic solution, can be a solution to restore or improve impaired vision. The impaired vision can be due to damaged transparency of the vitreous which is susceptible to several afflictions such infections, injuries, bleeding, particularly from diabetic retinopathy, blood vessels growing into the vitreous, again due to diabetes etc ... or to retinal detachment, an occasion wherein the retina is pulled into the vitreous, due to disease in the vitreous, small tears in the retina that allow liquid to seep behind it and push it forward, or injury to the eye that simply breaks the retina loose. In such case vitrectomy may be necessary to remove the vitreous in order to replace the retina and restore vision. Stabilised microplasmin or other stable small molecular weight protein comprising the catalytic domain of plasmin of present invention may be used under stabilised form or used after activation for pharmacological vitrectomy or to assist mechanical vitrectomy or vitreoretinal surgery.

Initially vitreous detachment was carried out by surgery without enzymatic aid. Such surgeries require a high level of skill in the practitioner to avoid permanent damage to the retina, and is occasionally accompanied by retinal tears, retinal detachment which may be complicated by proliferative vitreo-retinopathy. Each year, surgeons perform an estimated 200 000 vitrectomies in the Unites States and world-wide about 500 000 to correct devastating conditions including retinal detachments, macular degenerations, and diabetic eye diseases.

Accordingly, it was a goal of ophthalmologists to develop an alternative to surgical detachment of the vitreous. Knowledge of the molecular components of the vitreous, the vitreoretinal junction and the internal limiting membrane provided guideposts for the development of prior art for non-surgical methods of posterior vitreous detachment. Enzymes are now considered  
5 for use either to induce liquefaction of the vitreous or to weaken the adhesion of the vitreous cortex.

For example, U.S. Pat. No. 5,292,509, March 8, 1994, describes a method of detaching the  
10 vitreous body, the epiretinal membranes and/or fibrocellular membranes from the neural retina, ciliary epithelium and posterior lens surface of the mammalian eye, by injection of a protease-free glycosaminoglycanase, preferably chondroitinase ABC, into the vitreous cavity to degrade chondroitin sulphate glycosaminoglycan/proteoglycan, a component responsible for vitreoretinal adhesion. Chondroitinase would permit complete disinsertion of the vitreous  
15 body and/or epiretinal membranes. A method is claimed for disinserting the ocular vitreous body, epiretinal membranes or fibrocellular membranes from the neural retina, ciliary epithelium and posterior lens surface of the mammalian eye as an adjunct to vitrectomy which comprises administering to said eye an effective amount of an enzyme which disrupts or degrades chondroitin sulfate proteoglycan localized specifically to sites of vitreoretinal  
20 adhesion and thereby permit complete disinsertion of said vitreous body and/or epiretinal membranes.

Other investigators have concentrated on the collagen component of the vitreous on the  
25 hypothesis that collagen fibrils attach the vitreous to the retina. O'Neill and Shea (Canad. J. Ophthal. 8:366, 1973) described the use of bacterial collagenase (1.2 µg-1.0 mg) injected directly into the vitreous and observed the effects on the fibrillar structure of the vitreous after 6 to 17 days. The collagenase injection resulted in disruption of the fibrillar structure in the vitreous, and the internal limiting membrane, as well as disruption of the outer layers of the  
30 retina. To minimise the side effects of vitreous liquefaction and retinal damage, the authors suggested using larger doses of the bacterial collagenase enzyme, up to 5 mg, for 24 to 72 hours.

Moorhead et al (1980) used injection of purified collagenase (Clostridiopeptidase A) for preretinal cicatricial. A 30 minutes incubation in the rabbit eye did digest vitreal scar tissue while not altering the retina morphology. Light and transmission electron microscopy were used to determine effects on the inner limiting membrane and retinal ganglion and Muller's cells and to evaluate enzyme digestion of preretinal scarring. Removal of the injected collagenase by vitrectomy resulted in normal electroretinograms and retinal morphology 48 hours postoperatively Moorhead,-L-C; et al Arch-Ophthalmol. 1980 Oct; 98(10): 1829-39. Moorhead was by our knowledge the first to propose that enzymes like collagenase could represent a new approach to vitrectomy.

Later, Mandl,-I (1982) used a bacterial collagenase (from *Clostridium histolyticum*), a metalloproteinase capable of cleaving native collagen types I, II, III, IV and V, as an adjunct in vitrectomy (Arzneimittelforschung. 1982; 32(10a): 1381-4) and Moorhead et al. (1983) describes the enzyme-assisted vitrectomy with Clostridiopeptidase A. This bacterial collagenase caused extensive digestion of scar tissue after incubation periods of 10, 15, and 30 minutes without morphologic damage to cicatricial cellular elements or to the inner limiting membrane of the retina. He proposed the use of collagenase as an adjunct to vitrectomy (Moorhead,-L-C; et al, Arch-Ophthalmol. 1983 Feb; 101(2): 265-74). Lateron, Moorhead,-L-C and Radtke,-N.(1985) used Clostridiopeptidase A, in a clinical set up without side effects of lens opacity, lens dislocation, or retinal haemorrhage to assist vitrectomy with membrane stripping in six patients with dense intravitreal fibroproliferative tissue associated with retinopathy of prematurity, diabetic retinopathy, or proliferative vitreoretinopathy (Moorhead,-L-C and Radtke,-N., Retina. 1985 Spring-Summer; 5(2): 98-100).

Tezel,-T-H; et al. (1998) induced a posterior vitreous detachment (PVD) in porcine and human cadaver eyes in vitro by injecting into the vitreous cavity of enucleated porcine (0.05-25 U/mL) and human (5 U/mL) eyes of dispase, a bacillus-derived neutral metalloprotease. They reported that dispase cleaved the attachment of the posterior hyaloid to the internal limiting membrane with minimal damage to the inner retina. Retina were seemingly similar for dispase-treated and control eyes (Tezel,-T-H; et al, Retina. 1998; 18(1): 7-15). Based on examples with Dispase, Kaplan, Henry J. and Tezel, Tongalp H. (March 3, 1998, US5722428) describe a method for treating a subject to promote a posterior vitreous detachment comprising introducing into a vitreous cavity of an eye of a subject in need of such treatment an enzyme

which specifically cleaves ~~type IV collagen and fibronectin in an amount~~ effective to promote a posterior vitreous detachment. Since dispase has proteolytic activity against type IV collagen, a concern that this agent might adversely affect the ILL of the retina, which is actually the basal lamina of retinal Mueller cells and is composed of type IV collagen closely associated with glycoproteins. Yet, the histologic studies in porcine and human cadaver eyes have demonstrated that the lamina rara externa of the ILL was affected, with lesser effect on upon the lamina densa (Tezel TH et al. Retina 1998, 18: 17 - 25).

Another enzymatic treatment of the eye was induction liquefaction of vitreous humor to prevent eye disorder in mammal - comprises contacting vitreous humor with hyaluronidase to liquefy vitreous humor (Aragon G A C; et al Nov. 7, 2002 WO9852602A). Hyaluronidase is not likely to induce vitreoretinal separation, but is has been proposed for the clearance of vitreous haemorrhage without vitrectomy. However in the phase III FDA administration trial this drug was not found to be effective.

Note plasmin does appear to cause some limited inflammatory response in humans. It is likely that many of these compounds can. For pharmacologic vitreolysis endogenous human derived agents without considerable immunogenicity or antigenicity or which will not considerably modulate the immune effector mechanism might be desirable to bacterial proteases. Immune activation in the eye could promote the development of proliferative vitreoretinopathy (PVR) which could lead to a complex re-detachment of the retina. PVR is one of the major adverse events in vitreo-retinal surgery. Furthermore, if a given substance was antigenic, it would limit its potential re-use in a given patient. Hence, the compound could only be used in one eye, while many ocular processes are bilateral. Such endogenous protease is for instance human plasmin, a endogenous serine protease mediating fibrinolysis, which had been found to have properties to hydrolize a variety of glycoproteins, including laminin and fibronectin (Liotta LA, et al. Cancer Res 1981; 41: 4629-4636 & van Setten G. B. et al Curr Eye Res 1989 Dec. 8 (12): 1293-8), which are major adhesion glycoproteins at the retinovitreal interface.

Moreover, plasmin has a biochemical and biophysical relevance in endogenous processes of the eye. It had for instance been demonstrated, as early as 1980, that the plasminogen activator - plasmin system plays an endogenous role in the cornea of eyes (Berman M et al, Invest-Ophthalmol-Vis-Sci. 1980 Oct; 19 (10): 1204 - 21). Moreover, Immonen I. J. et al

demonstrated that active plasmin (1.0 to 15.2 micrograms/ml) is present in subretinal fluid in eyes with rhegmatogenous retinal detachment. Also, a plasmin-inhibitor complex was demonstrated in some eyes, providing evidence of a physiological role of plasmin in the human eye. The plasmin system was activated in eyes with retinal detachment (Immonen I.J. *Acta Ophthalmol* 1988 Dec. 66 (6) 647 – 51 & Immonen I. et al *Curr Eye Res* 1989 Mar, 8 (3): 249 – 52). Van Setten et al. (1989) demonstrated, for instance, a role of eye tear fluid plasmin and plasminogen activator in eye tear fluid in corneal wound healing. Plasmin (Pm) is the natural enzyme that is specific to fibrin, and thus can act to dissolve blood clots in vivo. Plasminogen (Pg) is an important compound of mammalian blood on activation by urokinase or streptokinase, the Pg molecule converts to a double chain form called plasmin (Pm). The structure, modification, and method of functioning of plasminogen/plasmin have been extensively studied. Human plasminogen has been fully sequenced and the positions of the disulphide bonds established. (See Sottrup-Jensen et al. (1978), in *Atlas of Protein Sequence and Structure*, eds. Dayhoff (National Biomedical Research Foundation, Silver Spring, MD), Vol. 5, suppl. 3, p. 91.). Human Pg contains 790 amino acids in one polypeptide chain. On activation, the peptide bonds between Arg560 and Val561 and Lys76 and Lys77 are cleaved. As a consequence plasmin (Lys77 -Pm) contains two polypeptides, a heavy A chain of 484 amino acids connected by two disulphide bonds to a light B chain of 230 amino acids. Native Pg can be recovered in two glycosylated forms, F-1 and F-2 possessing one or two glycosylations, respectively...” (Castellino (1981) (*Chemical Reviews*), 81:431, at 432-433). The measured molecular weight of human Pg is 92,000 to 94,000. Plasmin acts on Pg to cleave 76 residues between the 76 and 77 position lysines. The trimmed molecule is known as Lys77 -Pg, or when activated as Lys77 -Pm.

The above mentioned findings gave impetus to the development of plasmin as an agent to induce posterior vitreous detachment. Trese Michael T. et al (1994) suggested removing the vitreous humor from an eye by introducing plasmin into the vitreous humor in order to induce posterior vitreous detachment. Thereafter, the vitreous should be removable by conventional methods and replaced by a sterile saline solution. Trese's disclosure, however, did not teach the procedures on the preparation of the plasmin composition. It merely an invitation to experiment, with no assurance of success and suggestion that injection and removal of plasmin are necessary steps. It was stated that although the precise amount of plasmin necessary to

induce the posterior vitreous detachment is unknown, it has been found to be between one unit and three units (Trese Michael T. et al. April 19, 1994 US 5,304,118).

By our knowledge it were Verstraeten T. C. et al. (1993), who demonstrated for the first time  
5 by experimentation that plasmin (1 U) injected into the vitreous of rabbit can induce posterior  
vitreous detachment, demonstrating that it is a useful biochemical adjunct to mechanical  
vitrectomy. Plasmin produced a cleavage at the vitreoretinal interface by hydrolysing  
glycoproteins, including laminin and fibronectin, which are found at the vitreoretinal junction.  
By degrading these links between these components of the vitreoretinal interface and the inner  
10 limiting membrane (ILM), therapeutic posterior vitreous detachment (PVD) had become  
possible. Plasmin treatment performed with or without subsequent vitrectomy (Verstraeten et  
al. Arch. Ophthalmol. 11:849 – 854, 1993). Gandorfer et al. (2001) demonstrated complete  
vitreoretinal separation by cleavage between vitreous cortex and inner limiting membrane after  
injection of 2 U plasmin in 0.1ml in post mortem pig eyes, the retinal morphology being intact  
15 but with sparse collagen fibrils covering the inner limiting membrane. This was confirmed on  
human cadaver eyes Plasmin (2 U/0.1 ml) induced complete vitreoretinal separation with  
sparse collagen fibrils covering the inner limiting membrane without morphologic alteration of  
the retina (Gandorfer et al. Am J Ophthalmol. 2002 Jan., 1333 (1) 156 – 9) or by Li Xiaoxin et  
al et al. 2002 who demonstrated that 3 U plasmin could seemingly induce complete posterior  
20 vitreous detachment by degrading adhesion glycoproteins such fibrinectin (FN) and laminin  
(LN) (Li Xiaoxin et al Graefe's Arch Clin Exp. Ophthalmol 2002 Jan 240 (1) 56 – 62).  
However, so far all clinical interventions with plasmin to assist vitrectomy and macular hole  
closure could only be done with autologous plasmin enzyme (APE), said plasmin isolated from  
the patient's own serum before use at surgery. Such APE generation constitutes several  
25 disadvantages. It is a laborious and costly method that has to be carried out in specialised  
coagulation laboratories and on the site of the treatment. The use of an external or  
heterologous source of small molecules comprising the catalytic domain of plasmin  
(LMWPP's) such as recombinant micro-Pm or recombinant mini-Pm can thus reduce treatment  
time of patients, expense and patient/medical staff inconvenience. Successful clinical use of  
30 autologous plasmin enzyme (APE) to facilitate the formation of posterior vitreous detachment  
has been demonstrated by Chow, D.R. et al 1999 and for r closure of stage 3 macular holes by  
Trese T. et al., 1999 (Chow, D.R. et al Retina No. 5 1999 p 405 – 9 & Trese T. et al., 1999.  
Annual Meeting of the Association for Research in Vision and Ophthalmology Fort

Lauderdale (IOVS), Florida, May 9 - 14, 1999). ~~Macular hole closure could be obtained in~~  
patients treated with 0.4 IU of APE (Trese et al. Ophthalmology 2000 Aug. 107 (8)). APE  
has also been tested in patients as an adjunct to vitreous surgery in eyes with advanced diabetic  
retinopathy (Williams, J.G. Ophthalmology Vol 108, No 10, 2001 Oct., 1607 - 11.).

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Another drawback to treatment of eye disorders by plasmin (Pm) is its large molecular weight.  
The measured molecular weight of Pm is from 65,000 to 83,000. The diffusion in the vitreous  
and towards the vitreoretinal interface of such large molecules is hindered. Smaller molecules  
still comprising the catalytic activity of plasmin are thus desirable for the treatment of diseases  
or dysfunctions in the eye. Moreover, plasmin is a serine protease with broad specificity. APE  
of the prior art, APE, beside being only derivable from plasma or serum of the patient to treat,  
is highly prone to degradation and can thus not be stored for prolonged periods prior to use.  
This is a serious limitation for clinical use, challenging a safe and standardised treatment of eye  
disorders. Stabilised, low molecular weight molecules with the catalytic domain of plasmin  
LMWPP's are a solution to this problem.

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Present invention involves the use small molecule or low molecular weight molecules  
comprising the catalytic domain of plasmin LMWPP's, which are not necessarily autologous  
and may be obtainable by recombinant production such as miniplasmin (mini-Pm) or  
microplasmin (micro-Pm), and preferably stabilised miniplasmin (s-mini-Pm) or microplasmin  
(s-micro-Pm) to treat eye disorders such as proliferative diabetic retinopathy, age-related  
macular degeneration, amblyopia, retinitis pigmentosa, macular holes, macular exudates,  
cystoid macular oedema in a subject in need thereof, by a method comprising administering to  
said subject a miniplasmin or a microplasmin in an amount sufficient to prevent, inhibit or  
remove the pathological eye condition or sequelae of the pathological eye condition. It further  
involves pharmacological vitrectomy by non autologous or recombinant stabilised miniplasmin  
(s-mini-Pm) or stabilised microplasmin (s-micro-Pm) without removal of said mini-Pm or said  
micro-Pm from the eye. The mini-or microplasmin, preferably stabilised mini-or microplasmin,  
can be delivered by intravitreal injection or by ocular administration. At least the recombinant  
micro-Pm of present invention has been demonstrated to be effective in inducing posterior  
vitreous detachment or separation of retina and vitreous at the vitreoretinal interface and to be  
a means to micro- and ultrastructural change of the vitreous. The fibrillar structure is modified  
to a more amorphous, ground glass consistency, with lower viscosity, which demonstrates

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liquefaction of the vitreous body. Simultaneous liquefaction and vitreoretinal dehiscence without causing toxic damage to the eye, as has been demonstrated by recombinant microplasmin, is a combined feature that is important for successful treatment of eye anomalies such as vitreo-papillopathies, vitreomaculopathies and peripheral retinal traction. Liquefaction  
5 of the vitreous gel and vitreoretinal dehiscence, without using combinational therapy (e.g. treatment of hyaluronidase and dispase) may be an advantage.

Such small molecule or low molecular weight molecules comprising the catalytic domain of plasmin LMWPP's may be a microplasmin (micro-Pm), having molecular weights as  
10 determined by gel electrophoresis of about 26500 Da in reduced form and 29000 Da in non-reduced form (in the range of 26000 to 29000 Da) and being obtainable by autolytic reaction of plasmin and plasminogen, which reaction is promoted by high alkaline pH's or other reaction methods as described by Hua-Lin Wu and Guey-Yueh Shi, Sept 27, 1988 US4774087. The calculated molecular weights from the known amino acid sequence of human Lys530 -Pm is  
15 28,635, and of Leu531 -Pm was 28,507, thus in the range of 28000 Da to 29000 Da (Hua-Lin Wu and Guey-Yueh Shi, Sept 27, 1988 US4774087). Micro-Pm or Mini-Pm with molecular weight considerably less than plasmin can be obtainable by activation of microplasminogen or miniplasminogen by a plasminogen activator such as streptokinase or staphylokinase. Miniplasminogen is a derivative of plasminogen lacking the first four kringles, while  
20 microplasminogen is a derivative of plasminogen lacking the five kringles; both microplasmin and miniplasmin may be prepared by digestion of plasminogen with elastase and are fully activatable to microplasmin or miniplasmin, respectively. Miniplasmin has a molecular weight of 38000 Da and contains over 1 00 amino acids of the A chain including the fifth kringle structure. Mini-Pm and micro-Pm is also obtainable from recombinant produced  
25 miniplasminogen or microplasmin as described by Collen et al, June 2002 WO0250290.

Stabilised microplasmin or stabilised miniplasmin can be obtained by capturing substantially pure microplasmin or miniplasmin in a buffered acidic water (or additional stabilisers) said microplasmin or miniplasmin obtainable from a recombinant production process as for  
30 instance described in Collen et al WO0250290 or produced by an autolytic action of plasmin and plasminogen at high pH or other enzymatic cleavage methods as for instance described by Wu et al, US4,774,087. The activated proteins obtainable from microplasminogen or miniplasminogen, said microplasmin or miniplasmin, can be stabilised by means of a stabilising

agent preferably agents such as agent comprises an amino-acid ~~selected from the~~ group consisting of lysine, 6-amino hexanoic acid and tranexamic acid or by a stabilising medium preferably a stabilising medium such as an acid solution or an acid buffer such as an acid citrate buffer for example with a pH of about 3.39. Such stabilised miniplasmin or microplasmin may  
5 further be processed by lyophilization. However the man skilled in the art has several stabilisation agents and media available to stabilise said micro- or miniplasmin. It might comprise use of a low pH buffer selected from acetic acid, citric acid, hydrochloric acid, carboxylic acid, lactic acid, malic acid, tartaric acid, benzoic acid, serine, threonine, methionine, glutamine, alanine, glycine, isoleucine, valine, alanine, aspartic acid, derivatives or  
10 combinations thereof. Such buffer may contain further stabilisers such as a polyhydric alcohol, pharmaceutically acceptable carbohydrates, salts, glucosamine, thiamine, niacinamide, or combinations thereof. The stabilising salts can be selected from the group consisting of sodium chloride, potassium chloride, magnesium chloride, calcium chloride and combinations thereof. Sugars or sugar alcohols may also be added, such as glucose, inulose, mannitol, sorbitol,  
15 sucrose, lactose, trehalose, and combinations thereof.

#### ILLUSTRATIVE EMBODIMENT OF THE INVENTION

20 The following detailed description and the accompanying examples are provided for purpose of describing and explaining certain preferred embodiments of the invention only, and are not intended to limit the scope of the invention in any way.

In human beings, the anatomy of the eye includes a "vitreous body" which occupies  
25 approximately four fifths of the cavity of the eyeball, behind the lens. The vitreous body is formed of gelatinous material, known as the vitreous humor. Typically, the vitreous humor of a normal human eye contains approximately 99% water along with 1% macromolecules including: collagen, hyaluronic acid, soluble glycoproteins, sugars and other low molecular weight metabolites. The retina is essentially a layer of nervous tissue formed on the inner  
30 posterior surface of the eyeball. The retina lies on a retinal pigment epithelium under which a highly vascularized structure can be found called the choroid. The retina may be divided into an optic portion which participates in the visual mechanism, and a non-optic portion which does not participate in the visual mechanism. The optic portion of the retina contains the rods

and cones, which are effectual organs of vision. A number of arteries and veins enter the retina at its center, and splay outwardly to provide blood circulation to the retina. The posterior portion of the vitreous body is in direct contact with the retina. Networks of fibrillar strands extend from the retina and permeate or insert into the vitreous body so as to attach the vitreous body to the retina.

The preferred route of administration of said low molecular weight protein with catalytic plasmin domain (LMWPP), preferably said mini-Pm or micro-Pm is by intraocular injection directly into the vitreous body. The injection can be performed according to procedures standard in the art. Alternatively, however, the low molecular weight molecules with catalytic plasmin domain of the present invention may be administered by any other suitable route of administration (e.g., topically) which results in sufficient distribution of the enzyme(s) to the vitreous body to cause the liquefaction and vitreoretinal dehiscence effect.

The preferred injectable solution may contain, a micro-plasmin or other small molecular weight molecules with a catalytic plasmin domain to be administered intravitreally at doses selected from the range of 0.01 mg to 0.2 mg per eye and selected from a delivery volume range of 0.05 ml to 0.3 ml per eye and preferably about 0.125 mg in 0.1 ml per eye, along with inactive ingredients which cause the solution to be substantially isotonic, and of a pH which is suitable for injection into the eye.

Such solution for injection may initially have a pH suitable to stabilise the micro-plasmin or other small molecular weight proteins with a catalytic plasmin domain or may be lyophilized to a dry state and, thereafter, may be reconstituted prior to use. These formulation ingredients can initially be dissolved in sterile water, sterile filtered and subsequently lyophilized to a dry composition. The lyophilized composition can be packaged for subsequent reconstitution prior to use, in a suitable solvent such as sterile isotonic saline solution or balanced salt solution. Such balanced salt solution typically contains: 0.64% sodium chloride, 0.075% potassium chloride, 0.048% calcium chloride dehydrate, 0.03% magnesium chloride hexahydrate, 0.39% sodium acetate trihydrate, 0.17% sodium citrate dihydrate, sodium hydride/hydrochloric acid to adjust the pH, and as much water (q.s.) as necessary to bring the solution to the final volume for injection.

The invention concerns a method for production of a posterior vitreous detachment (PVD) and modification of the vitreous structure by introducing into the eye of a subject an effective amount of a LMWPP, preferably non autologous LMWPP, and yet more preferably a recombinant LMWPP, most preferably the LMWPP being a miniplasmin or a microplasmin.

- 5 The promotion of PVD and structural modification of vitreous by such enzymatic method allows a physician to induce efficient pharmacological vitrectomy treating an intraocular condition, and to avoid complications such as retinal tear or retinal detachment which frequently result as a consequence of said intraocular condition, as well as a complication of surgical vitrectomy performed to treat said condition.

10

- Since the LMWPP of present invention can be stabilised prior to use and is obtainable as a substantially pure molecule from a large production batch, they are devoid of the problems of collecting and preparing autologous plasmin per patient. LMWPP's can be produced in large batches of uniform activity. Consequently LMWPP's in contrast to autologous plasmin are
- 15 suitable for producing a standardised dosage form (in mg per eye). They can be used in simpler and safer patient treatment protocols resulting into controllable procedure, the control depending less on the skill of the individual practitioners. Such standardised protocols are required for drug registration by responsible authorities such as FDA and EMEA, allowing more controllable drug packaging and storage, eventually according to the good manufacturing
- 20 practice for subsequent reconstitution prior to use according the instructions of an approvable standardised insert.

- Enzymatic induction of a posterior vitreous detachment and induced liquification and/or decreasing viscosity of the vitreous by one agent, said low molecular weight micro-Pm or mini-
- 25 Pm according to the invention, also has the advantage of obtaining efficient liquefaction and vitreoretinal dehiscence, without using mixtures of vitreous liquifying agents and vitreous detachment agents.

- In preferred embodiments, the LMWPP of present invention is mini-Pm, preferably
- 30 recombinant mini-PM and most preferably a stabilised recombinant mini-PM. In yet another preferred embodiment of present invention the LMWPP of present invention is micro-Pm, preferably recombinant micro-PM and most preferably a stabilised recombinant micro-PM. However, any other low molecular protein comprising the catalytic domain of plasmin at a

dose effective to induce posterior vitreous detachment and ultrastructural modification of vitreous, preferably said protein being a recombinant protein and most preferably a stabilised recombinant protein with said catalytic plasmin domain.

The LMWPP can be suspended in any ophthalmologically acceptable carrier for introducing proteins into the eye of a subject. An ophthalmologically acceptable carrier is a substance which is nontoxic to the subject given the treatment and which also does not inhibit catalytic plasmin activity. Such ophthalmologically acceptable carrier is preferably a sterile diluent having a pH and osmolarity compatible with normal human vitreous.

- 10 The invention permits use of LMWPP's, preferably micro-PM, in connection with any intraocular surgery in which production of a PVD and/or vitreous liquefaction is beneficial to a subject. As used herein, "intraocular surgery" means surgery within the eye and encompasses surgeries for many different conditions. Intraocular surgeries in which the invention can be used include vitrectomy for macular hole surgery, macular pucker, vitrectomy for proliferative
- 15 vascular retinopathies, repair of a retinal detachment, prevention of a retinal detachment, subretinal surgery, submacular surgery and retinal transplantation. Other intraocular surgeries to which the invention is applicable will be known to those of skill in the art.

The invention can also be used for non-surgical treatment of complications associated with certain conditions of the eye. Conditions treatable by the invention include those conditions in which a retinal tear or a partial or complete retinal detachment can occur if left untreated. Such conditions include diabetic retinopathy, central vein occlusion, proliferative vitreal retinopathy and proliferative vascular retinopathy, and conditions leading to intraocular hemorrhage. Other conditions to which the invention is applicable will be known to those of skill in the art.

25

As used herein, an effective amount of a LMWPP is a dosage large enough to produce a PVD and/or decreased viscosity of the vitreous in a subject to which the LMWPP is administered. Generally, an effective amount the LMWPP can vary with the subject's age and condition, as well as the extent of the condition being treated, and can be determined by one of skill in the

30 art. The dosage can be adjusted by the individual practitioner in the event of any complication.

The active LMWPP of the present invention can include an ophthalmologically acceptable carrier, as defined above, which carrier is suitable for administration to a human or other

animal. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. Ophthalmologically acceptable compositions can routinely contain salts, buffering agents, preservatives, compatible carriers, and optionally other therapeutic ingredients. When used in ophthalmology, the salts should be ophthalmologically acceptable, but nonophthalmologically acceptable salts can be conveniently used to prepare ophthalmologically acceptable salts thereof and are not excluded from the scope of the invention. Such ophthalmologically acceptable salts include, but are not limited to those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, maleic, tartaric, citric, ascorbic, pantoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, formic, malonic, naphthalene-2-sulfonic, benzenesulfonic and the like. Also, ophthalmologically acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group. The components of the ophthalmological compositions are also capable of being commingled with the molecules of the present invention, and with each other in a manner such that there is no interaction which would substantially impair the desired pharmaceutical affect.

A variety of administration routes for the LMWPP's are available. The particular mode selected will depend of course, upon the particular subject, the severity of the condition being treated and the dosage required for therapeutic efficacy. The methods of this invention, generally speaking, can be practiced using any mode of administration that is ophthalmologically acceptable, meaning any mode that produces effective levels of the active compounds without causing clinically unacceptable adverse affects. Preferably the mode of administration is injection.

The compositions containing the LMWPP can be prepared by any of the methods well known in the art of pharmaceutical industry according to the rules of Good Manufacturing Practice of product generation with for instance consistency runs, batch processing and storage and Good Manufacturing Practice packaging and with an insert teaching the medical practitioner in order to obtain a product suitable for over the counter sales and for in hospital pharmacies.

The LMWPP of the present invention can be in a bottle in an amount effective to promote a posterior vitreous detachment and ultrastructural modification of the vitreous when administered by an individual practitioner to a vitreous cavity of an eye. Preferably the LMWPP contained in the bottle is stabilised recombinant micro-Pm, preferably stabilised by  
5 manitol and citric acid and preferably lyophilised. The bottle can be formed of any material which does not inhibit the activity of the LMWPP contained therein. For example, the bottle can be made of glass or plastic. The bottle can have a piercable septum through which the active composition can be removed. In use, the septum of the bottle is pierced by the needle of a syringe, the LMWPP in ophthalmologically acceptable carrier solution is removed by syringe  
10 from the bottle and injected into the eye. The bottle preferably contains LMWPP at a concentration between about 0.5 mg / ml to 2 mg / ml and more preferably it will contain a lyophilised LMWPP, yet more preferably 1 mg or 5 mg per vial for reconstitution by a suitable medium such as balanced salt solution (e.g. BSS+).

## 15 SUMMARY OF THE INVENTION

Present invention involves the use of a LMWPP, preferably a stabilised LMWPP, more preferably a stabilised miniplasmin, and most preferably a stabilised recombinant microplasmin, to treat an eye disorder by inducing PVD and decreased viscosity of the vitreous.

20

Preferably the method of stabilised miniplasmin (s-mini-Pm) or stabilised microplasmin (s-micro-Pm) or their recombinant form for the treatment an ocular disorder, including but not limited to proliferative diabetic retinopathy, age-related macular degeneration, amblyopia, retinitis pigmentosa, macular holes, macular exudates, cystoid macular oedema, thickened  
25 hyaloid, retinopathy of prematurity in a subject in need thereof, by a method comprising administering to said subject a miniplasmin or a microplasmin in an amount sufficient to prevent, inhibit or remove said pathological eye condition or prevent, inhibit or remove sequelae of said pathological eye condition. The stabilised mini-Pm or micro-Pm may eventually be activated juts prior to the treatment or readily be administrated in a stabilising  
30 buffer with low buffering capacity.

## EXAMPLES

### **Example 1 General materials and methods**

#### ***General preparatory methods of microplasmin***

- 5 Recombinant human microplasmin produced in *Pichia pastoris* system as described in Collen et al, June 2002 WO0250290 was used. This microplasmin, in contrast to autologous enzymes derived from the to be treated patient, provides consistency in activity and can be dosed in weight rather than units of activity. The recombinant microplasmin was stabilised as in Collen et al, June 2002 WO0250290, and lyophilised 1 mg or 5 mg per glass vials. Reconstitution  
10 could be done by adding 7 BSS+ (Baxter) to obtain a final pH between 6,4 to 7,4.

### **Example 2 New fixation method**

- 15 This experiment was carried out to establish a fixation technique reliable to investigate the effect of media and agents on posterior vitreous detachment in normal porcine eye. The effect of time on normal porcine eyes was studied.

- The eyes were obtained fresh from slaughterhouse and either immediately processed or allowed to sit at room temperature for up to 6 hours. Consequently the eyes are fixed in  
20 Peter's solution at 0°C to stop the enzymatic reaction. Cornea removed to facilitate fixation. Slow dehydration.

- With up to 6 hours, no significant change in the ultrastructure of the retina, and vitreous remains attached to the retinal surface. This current methodology provides thus non-traumatic  
25 fixation and preparation of eye tissue and minimises possibility that sampling artefact causes separation of vitreous from retinal surface. Whole retinal surface from the optic nerve to the periphery can be studied.

### **Example 3 : Porcine eyes at various concentrations at 24 degrees**

- 30 Freshly slaughtered pigs eyes were injected in the vitreous body with increasing doses of microplasmin starting with 0.0625 mg up to 0.39 mg in 0.1 ml (BSS+) pH range was 7.92 at 0.0625 mg up to 6.52 for 0.390 mg

On gross histology, at the higher dose of 0.39 mg microplasmin the retinal interface had a whitish appearance. On electron microscopy, there are multiple small elevations in the retinal structure which suggest localised retinal detachments. Lower doses demonstrate no ocular or retinal toxicity.

5

Example 4 : Comparison between micro-Pm and placebo treatment in time course.

The experiment was carried out on freshly enucleated pig eyes with immediate injection at room temperature (24°C). To determine ability of microplasmin to detach the vitreous, eyes were injected with 0.125 mg in 0.1 ml in the vitreous body. The buffer was BSS+

10

Detachment of vitreous was visible from about 60 minutes onwards. It was particularly present at 120 minutes in all sections of the retina except next to the ora serrata. On electron microscopy not only was the vitreous separated from the retina surface, but also the structure of the vitreous was changed with less fibrillary structure present. Retinal structure is unchanged. There was no sign of autolysis. Vacuolation of cells is often seen as an early sign of autolysis. This was not present

15

It can thus be concluded that microplasmin is capable at a dose of 0.125 mg/ml to cause a separation of the vitreous base (posterior hyaloid) or posterior vitreous detachment (PVD) with a clean retinal surface up to the ora. This at room temperature appears around 60 minutes and is complete around 120 minutes.

20

Discussion on the examples 2 to 4

In experimental use, microplasmin demonstrated to have a "safe" range of at least 0.06 to 0.2 mg if microplasmin in which it provides consistent separation of the posterior hyaloid without inducing ultrastructural changes in the retina (no structural toxicity). The posterior hyaloid separation is not only at the optic nerve but also all the way to the vitreous base, and leaves a clear smooth retinal surface on which no collagen fibers can be recognised. This has been evaluated at high electron-microscopic (EM) scanning (12000x), a magnification high enough to exclude the possibility of undetected fibers.

25  
30

At the vitreous base, the vitreous demonstrated to be relatively intact; Collagen fibers are present, but at low magnification, some areas appear devoid of vitreous at the very base of the ora serrata (normally a zone extending from the peripheral retina onto the ora is considered the vitreous base and provides strong adhesion to the vitreous put this parenthetical definition in at the first mention of ora serrata). Thus, it appears possible that the adhesion of the vitreous to the ora is somewhat weaker with microplasmin than with plasmin or other vitreolytic agents we may not want to make such a statement, since we have not compared plasmin/microplasmin from a dose-response standpoint.

We also examined other ocular structures with the electron microscope. Using the dose of 0.125 mg/0.1 ml, we did not find toxicity to the retina as indicated above. Furthermore, the ciliary processes supporting the lens appeared to be normal.

Ultrastructural studies suggest that microplasmin leads to a structural change in the vitreous. The fibrillar structure is modified to a more amorphous, ground glass consistency, which may indicate liquefaction of the vitreous body. On fresh pathological examination, the vitreous does appear more liquid, but the vitreous still retains some structure. This will lead to an easier removal using a vitreous cutter and the facilitation of elimination of intraocular blood.

#### Example 5 : Vitreoretinal morphology assessment of microplasmin treated human post-mortem eye

It was aimed to demonstrate the efficacy of microplasmin in inducing vitreoretinal separation, following microplasmin-induced posterior vitreous detachment.

25

Methods: A volume of 0.2 ml microplasmin was injected into the vitreous cavity of 13 human post-mortem eyes. The 13 fellow eyes received balanced salt solution (BSS) and served as controls. 1.25 mg of microplasmin was diluted with 4 ml (2 ml, or 1.5 ml) of BSS-PLUS to achieve a final concentration of 0.3125 mg/ml (0.625 mg/ml, or 0.9375 mg/ml). A total volume of 0.2 ml of these solutions was injected into the vitreous cavity, resulting in a final dose of 0.0625 mg (0.125 mg, or 0.188 mg) of microplasmin within the eye.

Intravitreal injection of microplasmin: A dose of 0.0625 mg microplasmin (pH 7.4) was injected into the vitreous cavity of two eyes. 0.125 mg microplasmin (pH 7.2) was injected into the vitreous cavity of 5 eyes. 0.188 mg (pH 7.2) was administered in 2 eyes. After incubation at 37°C for 30 minutes, the globes were placed in fixative and hemisected. Retinal specimens were obtained from the posterior pole, and were investigated using scanning and transmission electron microscopy.

0.125 mg microplasmin resulted in complete vitreoretinal separation, consistent with a bare inner limiting membrane. Only sparse collagen fibrils covered the inner limiting membrane. Regarding the ultrastructure of the vitreoretinal interface, 0.188 mg microplasmin did not show any differences compared to 0.125 mg microplasmin.

Following treatment with 0.0625 mg microplasmin, scanning electron microscopy revealed a posterior vitreous detachment with collagen fibrils covering the inner limiting membrane.

The retinal morphology of all microplasmin-treated eyes was unchanged. The ultrastructure of the inner limiting membrane was well preserved. There was no difference between microplasmin-treated eyes and controls.

It was found that all control eyes showed an attached cortical vitreous. Scanning electron microscopy demonstrated persistent cortical vitreous covering the inner limiting membrane. All microplasmin-treated eyes showed posterior vitreous detachment.

It can be concluded that microplasmin induces a cleavage between the vitreous cortex and the inner limiting membrane without morphological alterations of the retina. 0.125 mg of microplasmin is sufficient to induce complete vitreoretinal separation which is consistent with a bare inner limiting membrane without persistent cortical vitreous.

Table 1

Dose	No of eyes	Controls	Results treated eyes	Controls
0.0625	2	2	PVD, collagen fibrils	No PVD
0.125	5	5	PVD, bare ILM	No PVD

0.188	2	2	PVD, bare ILM	No PVD
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## FIGURES AND DRAWINGS TO THE APPLICATION

### 5 Fig. 1 a & b

1a Retinal surface of placebo treated eye

1b Retinal surface of the eye treated with 0.125 mg microplasmin

Human post-mortem study- looking strait on to the retinal surface (Figure 1a showing not a  
clean surface which is a placebo eye, and fig 1b showing a beautifully clean retinal surface in  
10 eye given microplasmin 0.125mg).

### Fig. 2

Figure 2 is the control showing the mid periphery where a structure of fibrillar fibers are seen  
15 at 1500x and 12000x. The fibrillar structure of the vitreous is clearly visible in both. The lower  
shows you how the vitreous after dehydration collapses on the retinal surface

### Fig. 3

20 Figure 3 shows retina in the mid periphery in a zone where the vitreous is removed the picture  
is at 3600x, there is only a very spares network of fibers on an otherwise smooth retinal  
surface. The other picture is taken of vitreous which was present in another zone on the retinal  
surface (possibly by simple apposition). Here a coarse granular structure is visible.

### 25 Fig. 4

Figure 4 shows the retina structures after exposure for 120 minutes to microplasmin. The  
ultrastructure is intact showing no significant vacuolation. In the second image, the retina  
surface also appears very smooth.

30

### Fig. 5

Figure 5 is a low mag image of the ora showing the ciliary processes, and what appears to be fairly adherent vitreous, at least along the pars plan. Vitreous is also partially adherent in the zone N P closer to the ora serata. The following image shows zone N where again the vitreous has been largely lost from the retinal surface.

5

**Fig. 6**

Figure 6 shows the zone P at different magnifications. In this location, the fibrillar structure of the vitreous is not evident though it still appears to be attached to the underlying layers.

10

**Fig. 7**

Figure 7 shows in the top left panel a low magnification image of mid peripheral retina after slow dehydration of a porcine eye treated with microplasmin (dose 0.125 mg/ml for 120 minutes). Most of the retinal surface is free of vitreous as shown in the other panels. In the center of this image, there is a vitreous strand present. We cannot say anything about its attachments at this magnification. It is likely that this vitreous is just collapsed vitreous content onto the retinal surface.

15

Panel B shows at 800 X an area of bare retina adjacent to a vessel. Few cells are seen on the retinal surface. The irregular surface overlies the vessel itself.

20

Panel C and D are magnifications of the retinal area in B showing a smooth retinal surface largely devoid of vitreous or cellular material. At 3600x only a few fibrillar strands are visible.

25

Panel H essentially shows the same findings at a more central retinal location, while panel G shows the coarse granular structure of the vitreous which has lost its fibrillar structure. This vitreous is inherently very different in appearance to that seen in control animals.

30

**Fig. 8**

Ciliary processes which are intact after 120 minute treatment with microplasmin

**Pharmacological Vitreolysis****CLAIMS**

1. A use of a LMWPP for the manufacture of a drug to treat dysfunctions in the eye of a  
5 subject.
2. A use of a LMWPP for the manufacture of a medicament to treat or cure a disorder of the  
eye of a subject.
3. A use of a LMWPP for the manufacture of a medicament to prevent that a subject  
develops a visual impairment.
- 10 4. A use of a LMWPP for the manufacture of a medicament to induce a cleavage between the  
vitreous cortex and the inner limiting membrane without morphological alterations of the  
retina of an eye of a subject.
5. A use of a LMWPP for the manufacture of a medicament to induce vitreoretinal dehiscence  
in the eye of a subject in need thereof.
- 15 6. A use of a LMWPP for the manufacture of a medicament to induce vitreoretinal dehiscence  
and decrease viscosity of the vitreous in the eye of a subject in need thereof.
7. A use of a LMWPP for the manufacture of a medicament to facilitate the dissolution of  
intraocular blood.
8. A use of a LMWPP for the manufacture of a medicament to treat or prevent sequelae of  
20 the eye.
9. The use of the LMWPP of any of the claims 1 to 8, wherein said LMWPP has a molecular  
weight between 10 000 and 40 000 dalton.
10. The use of the LMWPP of any of the claims 1 to 8, wherein said LMWPP has a molecular  
weight between 20 000 and 32 000 dalton.
- 25 11. The use of the LMWPP of any of the claims 1 to 8, wherein said LMWPP has a molecular  
weight of about 26 500 dalton in reduced form or of about 29 000 in non reduced form as  
determined by a gel electrophoresis.
12. The use of the LMWPP of any of the claims 1 to 8, wherein said LMWPP is miniplasmin
13. The use of the LMWPP of any of the claims 1 to 8, wherein said LMWPP is stabilised  
30 miniplasmin.
14. The use of the LMWPP of any of the claims 1 to 8, wherein said LMWPP is miniplasmin  
obtainable by recombinant production.
15. The use of the LMWPP of any of the claims 1 to 8, wherein said LMWPP is microplasmin

16. ~~The use of the LMWPP of any of the claims 1 to 8,~~ wherein said LMWPP is stabilised microplasmin.

17. The use of the LMWPP of any of the claims 1 to 8, wherein said LMWPP is recombinant microplasmin.

5 18. The use, according to any one of the preceding claims, wherein the subject to be treated is a mammal.

19. The use, according to any one of the preceding claims, wherein the subject to be treated is a human.

10 20. The use, according to any one of the preceding claims, wherein the subject is a diabetic patient.

21. Advertising media and material and information media and material having or giving information about the indications and utilities of a LMWPP, preferably microplasmin, described above, especially those described in the above claims.

15 22. A method of selling a LMWPP, preferably microplasmin, by giving information of about the indications and utilities of said LMWPP, preferably microplasmin, described above, especially those described in the above claims.

20 23. Use of a pharmaceutically effective composition for use in a therapeutical treatment of the eye of a mammal in need thereof, comprising a pharmaceutically effective amount of a compound which is selected from a group of compounds consisting of microplasmin, an active derivative of microplasmin, a physiologically tolerated salt of the microplasmin derivative, miniplasmin, an active derivative of miniplasmin and a physiologically tolerated salt of the miniplasmin derivative.

25 24. Use of a pharmaceutically effective composition for use in a therapeutical treatment of the eye of a mammal in need thereof, comprising a pharmaceutically effective amount of recombinant stabilised microplasmin or a pharmaceutically effective amount of recombinant stabilised miniplasmin.

30 25. Use of a pharmaceutically effective composition for use in a therapeutical treatment of the eye of a mammal in need thereof, comprising a pharmaceutically effective amount of recombinant stabilised microplasmin or a pharmaceutically effective amount of recombinant stabilised miniplasmin, wherein said miniplasmin or said microplasmin is stabilised by manitol and/or citric acid.

26. Use of a pharmaceutically effective composition for use in a therapeutical treatment of the eye of a mammal in need thereof, comprising a pharmaceutically effective amount of

recombinant stabilised microplasmin or a pharmaceutically effective amount of recombinant stabilised miniplasmin, wherein said miniplasmin or said microplasmin is stabilised by manitol and/or citric acid and lyophilised.

- 5 27. Use of a pharmaceutically effective composition for use in a therapeutical treatment of the eye of a mammal in need thereof, comprising a pharmaceutically effective amount of recombinant stabilised microplasmin or a pharmaceutically effective amount of recombinant stabilised miniplasmin, wherein said miniplasmin or said microplasmin is stabilised by manitol and/or citric acid, lyophilised and reconstituted by a balanced salt solution suitable for ophthalmologic treatment.
- 10 28. Use of the pharmaceutically effective composition of claims 23 to 27 for use in a treatment to prevent visual impairment of a subject.
- 15 29. Use of a pharmaceutically effective composition of claims 23 to 27 for use in a treatment of an ocular disorder, including but not limited to proliferative diabetic retinopathy, age-related macular degeneration, amblyopia, retinitis pigmentosa, liginous conjunctivitis, subretinal haemorrhage macular holes, macular exudates, cystoid macular oedema, thickened hyaloid, retinopathy of prematurity in a subject in need thereof, by a method comprising administering to said subject a miniplasmin or a microplasmin in an amount sufficient to prevent, inhibit or remove said pathological eye condition or prevent, inhibit or remove sequelae of said pathological eye condition.
- 20 30. A method for the treatment of an eye disorder in mammals, wherein an effective amount of a LMWPP is delivered to the eye to decrease the viscosity of the vitreous.
31. A method for the treatment of an eye disorder in mammals, wherein an effective amount of a LMWPP is delivered to the eye to induce vitreoretinal dehiscence.
- 25 32. A method for the treatment of an eye disorder in mammals, wherein an effective amount of a LMWPP is delivered to the eye to induce vitreoretinal dehiscence and to decrease the viscosity of the vitreous.
33. The method of claims 30, 31 or 32, whereby said LMWPP is selected from the group of compounds comprising a pharmaceutically effective amount of a compound which is selected from a group of compounds consisting of microplasmin, an active derivative of microplasmin, a physiologically tolerated salt of the microplasmin derivative, miniplasmin, an active derivative of miniplasmin and a physiologically tolerated salt of the miniplasmin derivative.
- 30

~~34. A kit containing a lyophilised and stabilised microplasmin for use in the method of claims~~

~~30, 31, 32 or 33 comprising a container with predetermined amount of stabilised LMWPP.~~

35. The kit of claim 34, wherein the LMWPP is stabilised by manitol and citric acid.

36. The kit of claim 34, wherein the LMWPP is stabilised by manitol and citric acid and

5 lyophilised.

37. The kit of claim 36, wherein the LMWPP is a microplasmin.

38. The kit of claim 36, wherein the LMWPP is a miniplasmin.

39. The kit of claim 36, 37 or 38, further comprising an ophthalmologic acceptable medium to reconstitute said lyophilised LMWPP.

10 40. The kit of claim 39, wherein said the reconstitution medium is a balanced salt solution.

41. The kit of any of the claims 34 to 39, further comprising an insert with information on said LMWPP and said method of any of the claims 30 to 33.

**Pharmacological Vitreolysis****ABSTRACT**

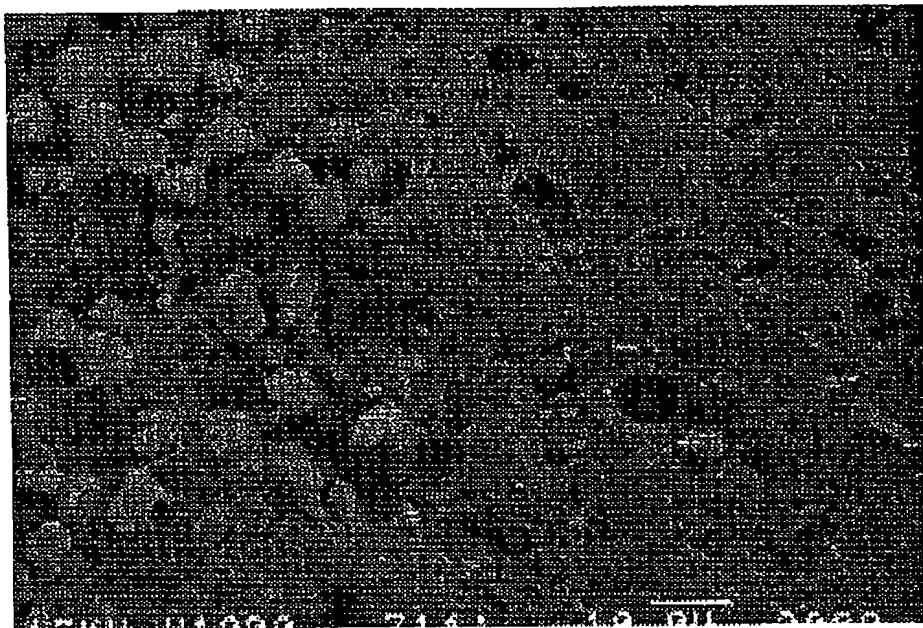
5

This invention relates to a method of treating diseases of the eye or dysfunctions in the eye. It also involves enzyme preparations for therapeutic administration to the eyes of humans or other mammals. Specifically, this invention is directed to a method and composition which involves stabilised microplasmin (s-micro-Pm) and/or stabilised miniplasmin (s-micro-Pm) useful in the liquefaction of the vitreous, a normally clear jelly-like substance that fills the eye from the iris to the retina, and/or to disinsert the peripheral vitreous from the neurosensory retina (pharmacological vitrectomy).

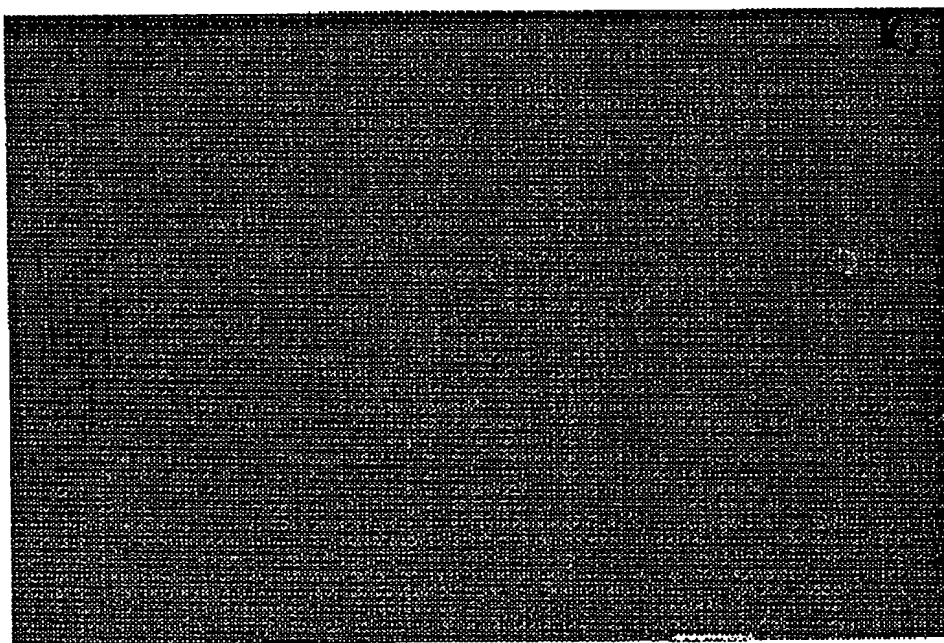
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**Fig 1**

**1A**

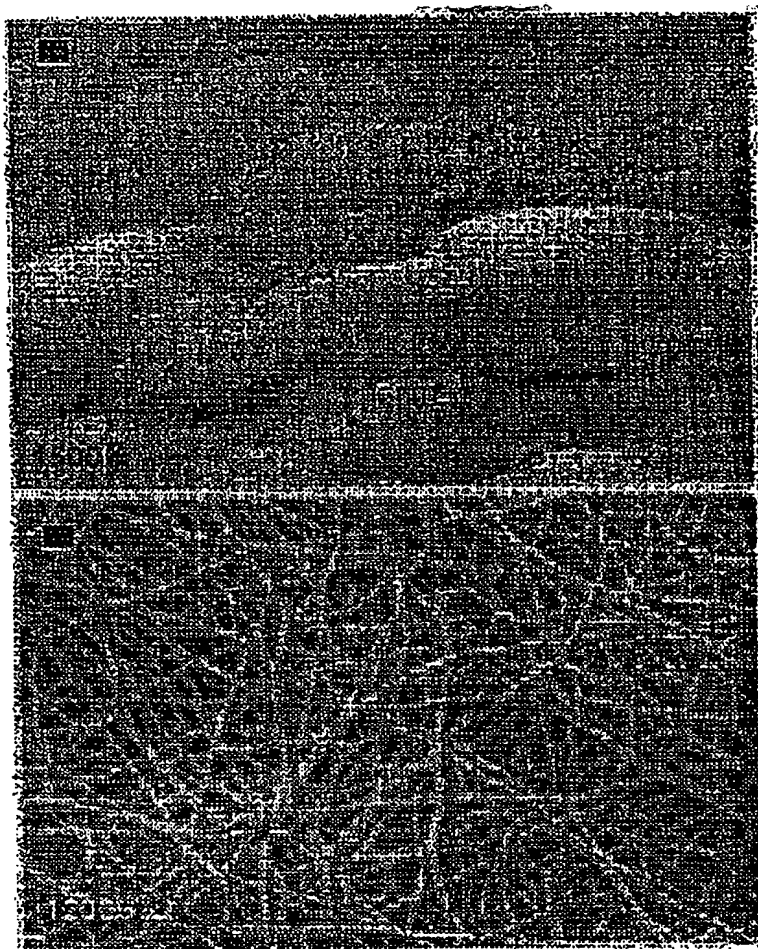


**1B**



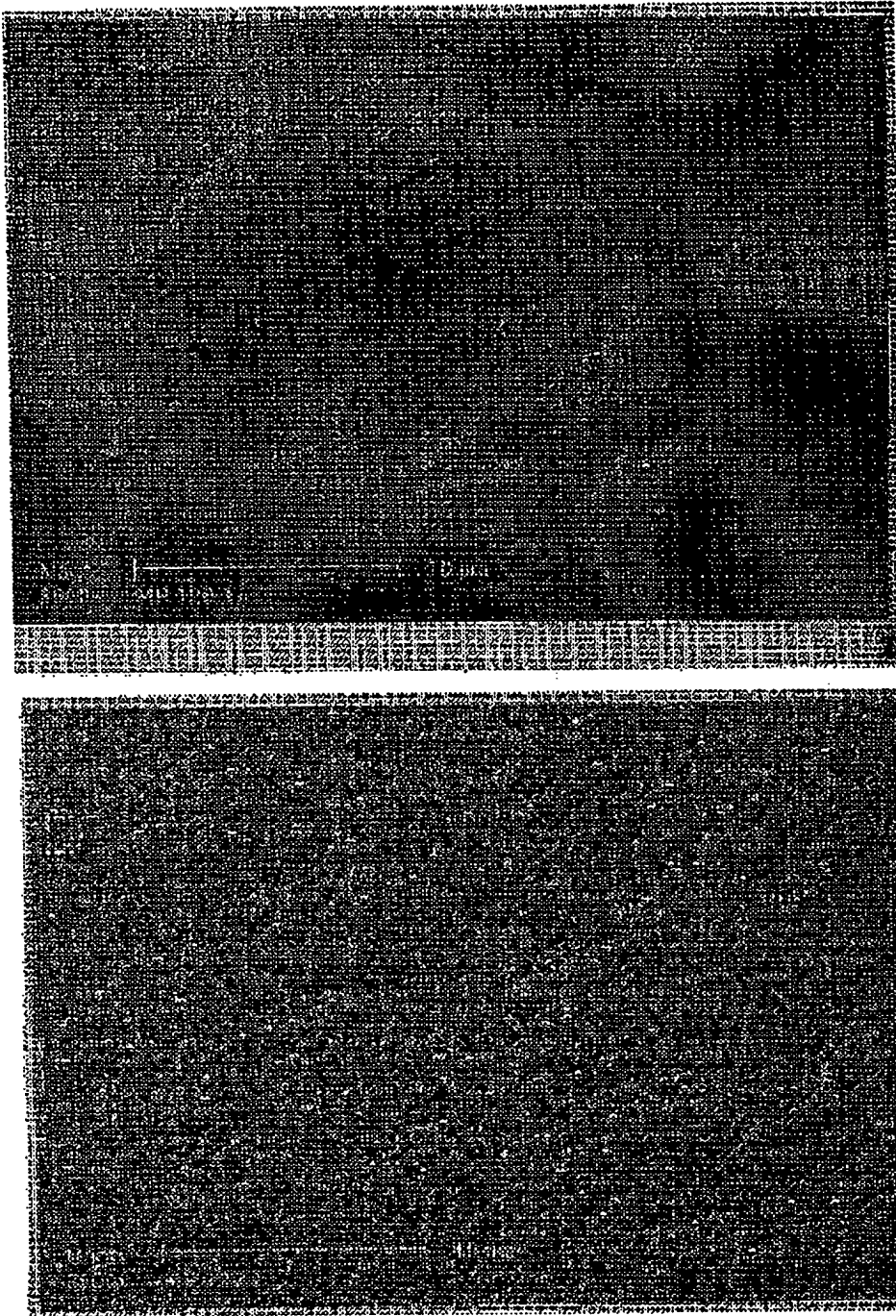
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Fig 2



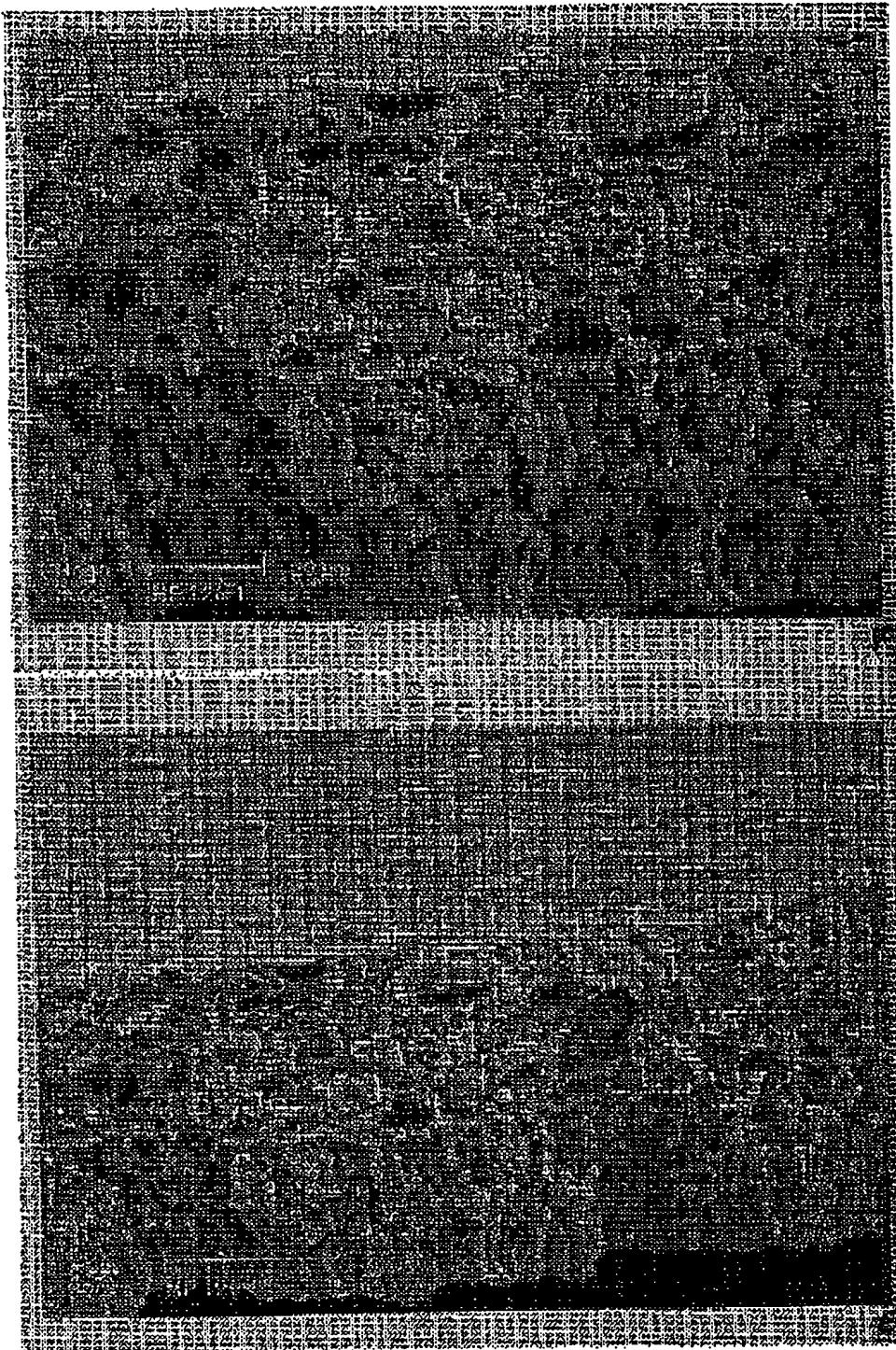
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**Fig. 3.**



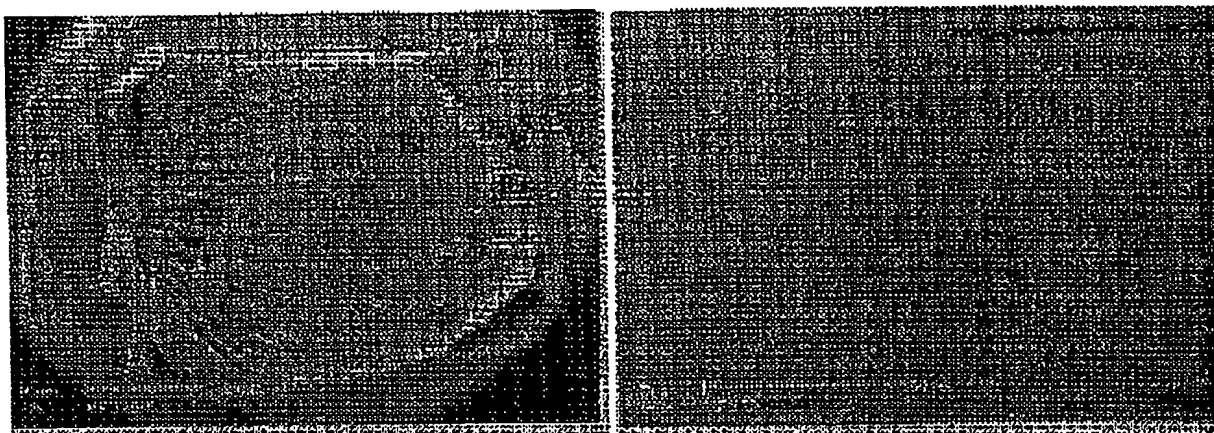
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Fig. 4



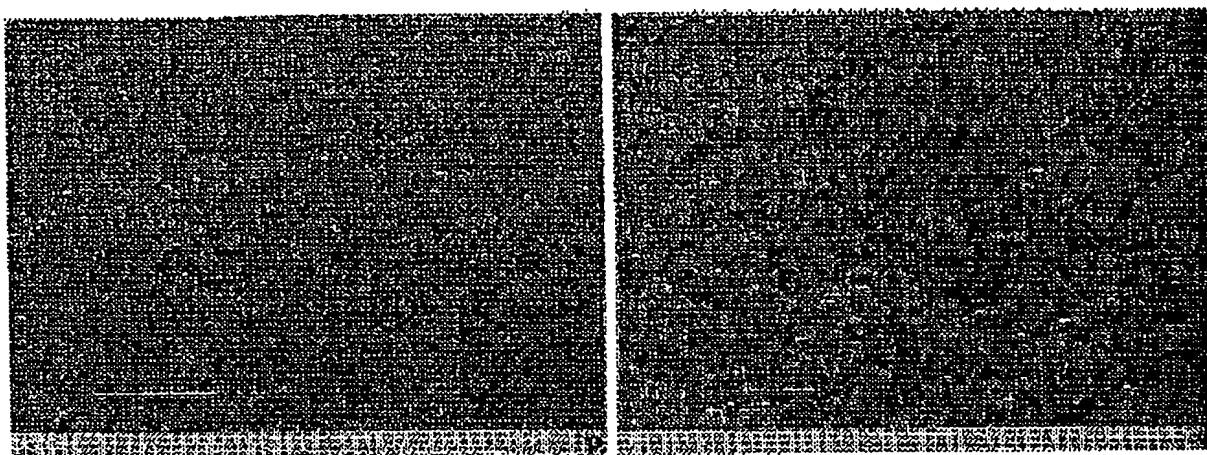
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Fig 5



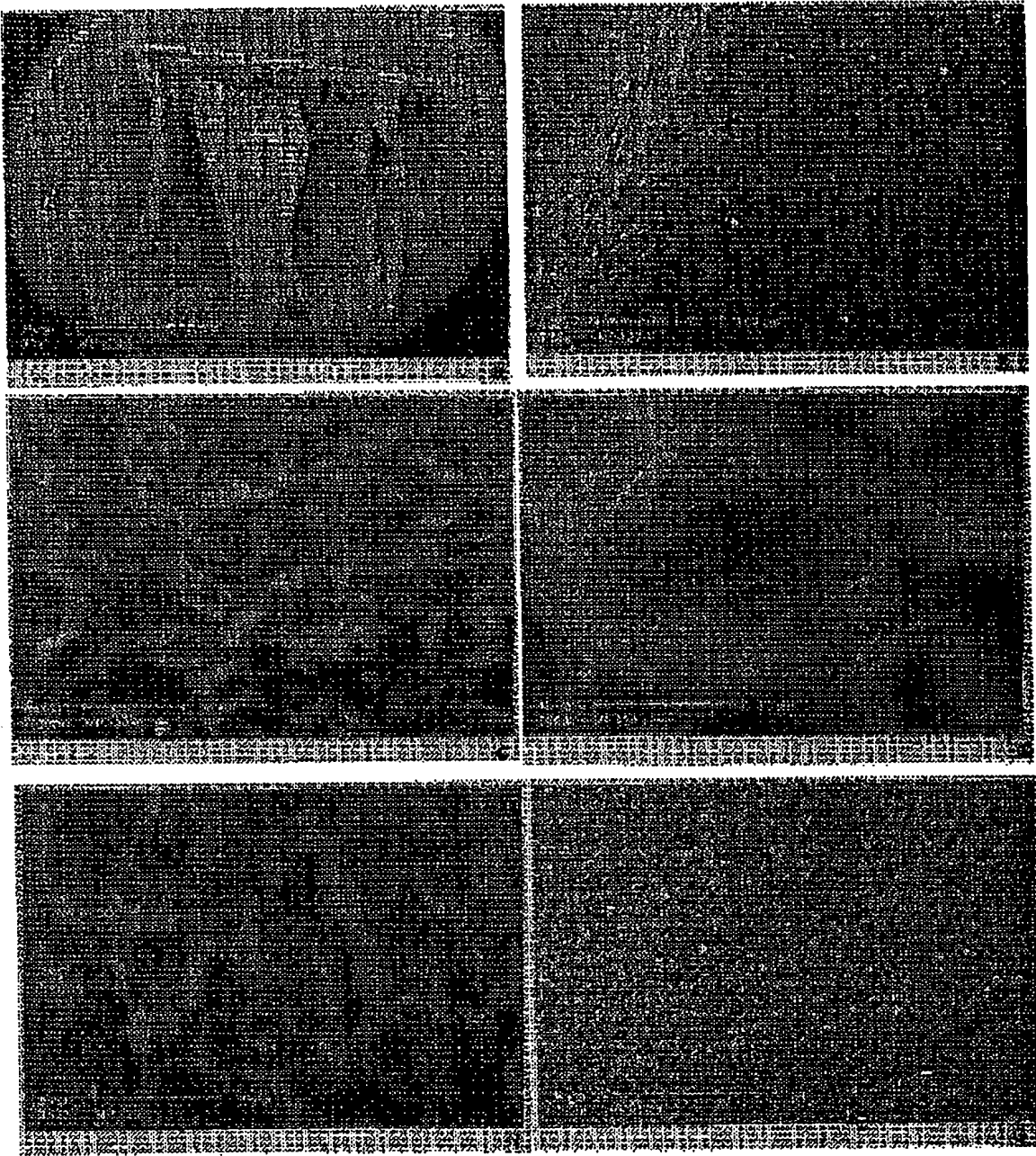
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**Fig. 6**



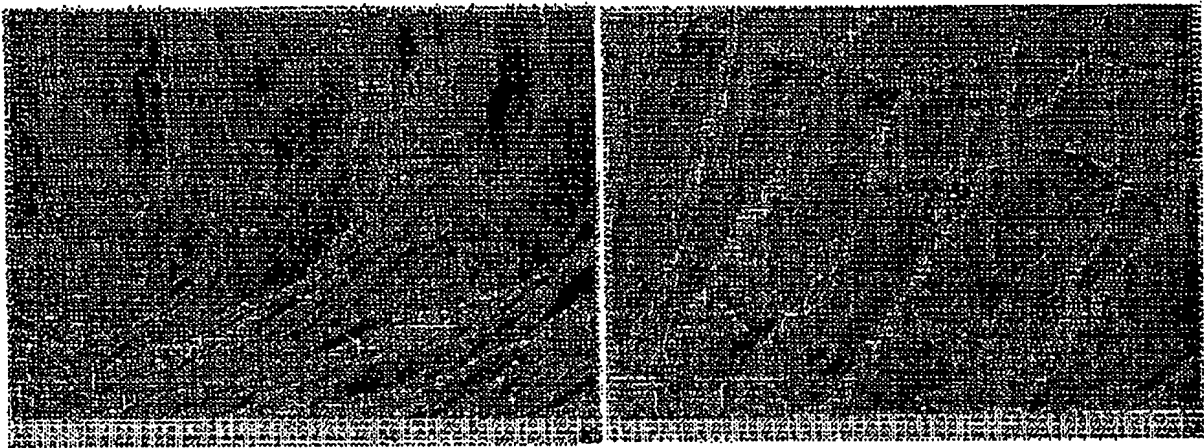
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Fig. 7



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Fig. 8



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